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The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

99200256.8

PRIORITY DOCUMENT
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Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

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Anmelder:
Applicant(s):
Demandeur(s):
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Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention:
Composition and method for obtaining specific immunisation against one or more antigens using different recombinant vectors

In Anspruch genommene Prioriät(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

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The original title of the application reads as follows:
PRODUCT AND METHOD FOR OBTAINING SPECIFIC IMMUNISATION AGAINST ONE OR MORE ANTIGENS.

Title: Product and method for obtaining specific immunisation against one or more antigens.

FIELD OF THE INVENTION

The invention lies in the field of medicine. More particularly the invention relates to vaccines, vaccine compositions and vaccination strategies for obtaining improved immune protection against antigens.

BACKGROUND OF THE INVENTION

The ultimate goal of developing prophylactic and/or therapeutic vaccines for a large number of infectious diseases has been difficult to achieve due to the inability to induce optimal immune responses to the pathogen in a safe and effective manner. The previously tried and proven approaches of vaccination with whole killed or live attenuated viruses are either unsafe or ineffective for the remaining infectious diseases of major public health concern. To avoid possible safety problems it has been possible to develop protein based vaccines consisting of one or several individual viral proteins or epitopes thereof. These are derived from individual viral genes expressed in vitro and purified as individual subunits in the protein in the absence of genetic material. Recombinant subunit vaccine approaches have proven effective for certain pathogens such as Hepatitis B. However, for many applications subunit antigens have been unsuccessful due to expression/production difficulties, alteration of relevant immunological epitopes or marked variability of the pathogen requiring the continuous development, fermentation and purification of new antigens.

Recombinant live viral or bacterial vaccine vectors were developed as potential solutions to some of these problems. A replicating live virus or bacteria which does not cause disease has the potential to be used as a vector. Attenuated viruses such as adenovirus, pox virus (i.e. vaccinia, MVA,

canary or fowlpox) or bacteria such as *E. coli*, are being developed and evaluated as live vectors. Due to their ability to replicate (in some cases in a limited fashion) in a host without serious side effects, makes them candidate to carry and express foreign genes as "vaccine" antigens. Recombinant vaccines have the advantage that they replicate in the host and thereby induce stronger immune responses than whole killed viruses or bacteria or subunit proteins. An additional advantage is that an immune response to an antigen encoded by said vector, may be improved by the stimulation of the immune system through the presence or the expression of additional proteins, for instance vector specific proteins for instance through providing adjuvant function. However, relatively few recombinant vector systems alone have been successful enough to be widely accepted for clinical use. Major problems other than safety have been pre-existing immunity in the case of vectors derived from infectious agents common in populations. Furthermore, subsequent immune responses against vector proteins themselves have created a further immunological barrier when more than one immunisation was required to boost responses to the recombinant vaccine antigen(s). One problem is that the immune system may mount an immune response against vector or vector encoded proteins together with an immune response against the antigen, designated the vaccination antigen, the immune response was intended to be directed toward in order to provide the host protection. The observation that the immune system may mount an immune response against a vector protein or a vector encoded protein creates a potential for competition for immune resources such as the availability of immune cells and/or cytokines, thereby lowering the desired response against vaccination proteins (see for example figure 1A). Another problem is the potential for more immunogenic antigens present in vector proteins or vector encoded proteins directing the immune response away from vaccination proteins. Additionally, immune responses against the vector eventually limit vector replication in the

host, thereby reducing the vectors intended purpose and effectiveness. A problem that specifically increases upon boosting of the immune response with the same or a similar vector or vector system. For instance, the use of different adenovirus serotypes comprising nucleic acid encoding similar vaccination proteins as vaccines is not optimal since the immune system will still be boosted against common antigens present in vector proteins and/or vector encoded proteins. A possible method to avoid this problem is to boost immune responses induced by the recombinant vectors with subunit protein. Several studies have shown that immune responses can be slightly improved by this method but that there is not an improvement in the ability of the vaccine to protect from infection.

SUMMARY OF THE INVENTION

The present invention provides novel means and methods for obtaining a specific immune response in an individual or animal. The invention further provides means and methods for decreasing the negative effects of vector proteins and/or vector encoded proteins while leaving desired effects, such as an adjuvant effect of said proteins at least in part intact (see for a non-limiting example the scheme depicted in figure 1B).

In one aspect the invention provides a product for vaccinating an animal or a human to obtain therein an immune response against at least one antigen, comprising at least two different vaccine compositions for sequential administration to said animal or said human, each containing at least said antigen or a precursor thereof, wherein at least two of said vaccine compositions differ from each other by the presence therein of a different vector.

In another aspect the invention provides a method for vaccinating an animal or human to obtain therein an immune response against at least one antigen, comprising

administering sequentially to said animal, at least two different vaccine compositions, each containing at least said antigen or a precursor thereof and wherein at least two of said vaccine compositions differ from each other by the presence therein of a different vector.

In yet another aspect the invention provides a use of an antigen, or a precursor thereof, for manufacturing a vaccine composition for vaccinating an animal or a human to obtain therein an immune response against said antigen, wherein said vaccine composition is administered sequentially with at least one other vaccine composition containing at least an immunogenic part, derivative and/or analogue of said antigen or antigen precursor, and a different vector.

DETAILED DESCRIPTION OF THE INVENTION.

In one aspect the invention provides a solution to circumvent the negative effects associated with vector proteins or vector encoded proteins in a vaccination procedure or a vaccine composition. To study problems associated with amplification of an immune response against vector proteins and/or vector encoded proteins a strategy was developed in which the use of different vector systems, to consecutively deliver the same or related antigen(s), was evaluated. The potential existed not only to substantially boost immune responses to the recombinant antigen, but to tailor the nature of the immune responses by priming and then delivering subsequent boosts to different immunological sites and/or antigen presenting cell populations. Indeed, the ability to induce preferred type-1 or type-2 like T-helper responses or to additionally generate specific responses at mucosal and/or systemic sites can be foreseen with such an approach.

The present invention provides means and methods for obtaining a specific immune response against at least one

antigen in an animal, in a vaccination procedure comprising a serial administration to said animal of at least two vaccine compositions comprising at least said antigen or a precursor thereof, by avoiding at least in part amplification of an immune response in said animal against vector antigens that may be present in one or more of said vaccine compositions or that may be encoded by nucleic acid present in one or more of said vaccine compositions or both. By at least in part avoiding said amplification of an immune response against vector antigens in said animal, potential masking of an immune response against said antigen is at least in part prevented. One method of avoiding at least in part an amplification of an immune response against vector antigens in said animal is to avoid at least in part the presence of vector antigens in said animal during said vaccination procedure. This may be achieved for instance by avoiding the presence of vector antigens in at least one of said vaccine compositions or by avoiding at least in part, expression of vector antigens encoded by a nucleic acid in a vaccine composition, or both. Preferably, amplification of an immune response in said animal or human against vector antigens is at least in part prevented by using for said serial administration of vaccine compositions, vaccine compositions comprising different vectors. Another preferred method of avoiding amplification of an immune response against vector antigens in said vaccination procedure is to use at least one vaccine composition useful for avoiding the presence of vector antigens in said animal and at least one vaccine composition comprising a vector. Preferably, when more then one vaccine composition comprising a vector is used, said vector in said vaccine composition is essentially different.

A process for vaccinating an animal or human may be any vaccination process provided that said process utilises serial administration of vaccine compositions containing at least an antigen or a precursor thereof, against which said animal or human should at least in part be vaccinated.

Vaccine compositions are preferably administered to said animal or human in an amount effective for eliciting an immune response in said animal or human. Vaccination does not necessarily need to result in complete protection against pathogens or cells comprising said antigen, a partial protection may also be a favourable result, for instance for weak immunogenic antigens.

Said antigen may be a complete protein or a part of a protein. Said antigen may also be a proteinaceous molecule, derived from nature or synthesised chemically.

In one embodiment of the invention said animal is a human.

In one embodiment the invention provides a product for vaccinating an animal or a human to obtain therein an immune response against at least one antigen, comprising at least two different vaccine compositions for sequential administration to said animal or said human, each containing at least said antigen or a precursor thereof, wherein at least two of said vaccine compositions differ from each other by the presence therein of a different vector.

Preferably said product comprises at least three of said compositions and wherein at least three of said vaccine compositions differ from each other by the presence therein of a different vector.

In one embodiment at least part of, said vector or a product thereof, functions as an adjuvant. An adjuvant in the context of the present invention is any molecule or combination of molecules, capable of modulating an immune response against said antigen. In one example an adjuvant has the capability to stimulate the immune system in said animal to elicit an immune response wherein said stimulation also stimulates the initiation or the amplification of an immune response against said antigen. In one example, an adjuvant is a classical adjuvant such as complete or incomplete freund adjuvant. In another example said adjuvant is a proteinaceous molecule immunologically different from said antigen, capable of eliciting an immune response in said animal or human.

Preferably said proteinaceous molecule comprises at least a functional part of a co-stimulatory molecule such as CD80, CD86, CD28, CD152, CD40 or CD40 ligand; of a cell-adhesion protein; of an immune response inhibitory protein; of an interleukin; of a major histocompatibility complex protein or of other proteins capable of modulating an immune response. An immune response may be modulated through at least in part inhibiting or preventing an immune response and/or at least in part inducing or enhancing an immune response.

In a preferred aspect of the invention vaccination is be performed together with a method for influencing at least in part immune system, for example in the direction of a preferred T helper 1 type of immune response or a more T helper 2 type of immune response. It is now widely accepted that T cell-dependent immune responses can be classified on the basis of preferential activation and proliferation of two distinct subsets of $CD4^+$ T-cells termed T_H1 and T_H2. These subsets can be distinguished from each other by restricted cytokine secretion profiles. The T_H1 subset is a high producer of IFN-γ with limited or no production of IL-4, whereas the T_H2 phenotype typically shows high level production of both IL-4 and IL-5 with no substantial production of IFN-γ. Both phenotypes can develop from naive $CD4^+$ T cells and at present there is much evidence indicating that IL-12 and IFN-γ on the one hand and IL-4 on the other are key stimulatory cytokines in the differentiation process of pluripotent T_H0 precursor cells into T_H1 or T_H2 effector cells, respectively, *in vitro* and *in vivo*. Since IFN-γ inhibits the expansion and function of T_H2 effector cells and IL-4 has the opposite effect, the preferential expansion of either IFN-γ producing cells (pc) or IL-4 pc is indicative of whether an immune response mounts into a T_H1 or T_H2 direction. The cytokine environment, however, is not the only factor driving T_H lineage differentiation. Genetic background, antigen dose, route of antigen administration,

type of antigen presenting cell (APC) and signalling via TCR and accessory molecules on T cells.
In a preferred aspect of the invention the immune system is directed toward a more T helper 1 or 2 type of immune response through using vectors with the property of modulating an immune response in one direction or the other.
In a preferred aspect of the invention at least part of said adjuvant function comprises means for directing the immune system toward a more T helper 1 or 2 type of immune response.

Preferably through using vectors with the property of modulating an immune response in one direction or the other. Examples of vectors with the capacity to stimulate either a more T helper 1 or a more T helper 2 type of immune response or of delivery routes such as intramuscular or epidermal delivery can be found in Robinson 1997, Vaccine 15:785-787; Sjolander et al 1997, Cell. Immunol. 177:69-76; Doc et al 1996, Proc. Natl. Acad. Sci. USA 93:8578-8583; Feltquate et al 1997, J. Immunol. 158:2278-2284; Pertmer et al 1996, J. Virol 70:6119-6125; Prayaga et al, Vaccine 15:1349-1352; Raz et al 1996, Proc. Natl. Acad. Sci. USA 93:5141-5145.

In one aspect at least one of said vectors comprises antigen presenting cells, preferably engaged in vivo but also in vitro from said animal. Preferably said antigen presenting cells are dendritic cells. Preferably said antigen presenting cells present said antigen, or an immunogenic part, such as a peptide, or derivative and/or analogue thereof, in the context of major histocompatibility complex I or complex II.

In a preferred embodiment at least one of said compositions comprises as an antigen precursor a nucleic acid encoding at least one proteinaceous molecule for inducing and/or boosting an immune response against said antigen. In a preferred embodiment said nucleic acid is capable of replicating in a cell of the animal or human being vaccinated. With the term boosting in this respect is meant amplifying an immune response such, that when said animal is

exposed to said antigen after the amplification, the immune response to said antigen is increased in magnitude compared to before said amplification. Said proteinaceous molecule for inducing and/or boosting an immune response against said antigen may be said antigen or an immunogenic part, derivative or analogue thereof. Alternatively, antigen or an immunogenic part, derivative or analogue thereof may be encoded by a nucleic acid present in said vaccine composition.

In a preferred embodiment said antigen is an antigen encoded by a nucleic acid of a pathogen, preferably of a virus, more preferably of a lentivirus or of a hepatitis C virus. In a preferred embodiment said antigen comprises at least an immunogenic part, derivative and/or analogue of a lentivirus *gag, pol, rev, tat, nef* or *env* protein or a combination thereof.

In a preferred embodiment at least part of said adjuvant function by a vector is provided by a nucleic acid which encodes at least one proteinaceous molecule capable of modulating an immune response. Preferably said nucleic acid is capable of replicating in a cell of the animal of the human being vaccinated. Preferably said proteinaceous molecule capable of modulating an immune response comprises a functional part of a co-stimulatory molecule such as CD80, CD86, CD28, CD152, CD40 or CD40 ligand; of a cell-adhesion protein; of an immune response inhibitory protein; of an interleukin; of a major histocompatibility complex protein or of other proteins capable of modulating an immune response.

In one embodiment the invention provides vaccine compositions wherein said vector is nucleic acid delivery vehicle comprising said nucleic acid. In a preferred embodiment said nucleic acid is capable of replicating in a cell of an animal or human being vaccinated. In a preferred embodiment said replicated nucleic acid has at least a limited capacity to spread to other cells of the host and

start a new cycle of replication and antigen presentation and/or present adjuvant function. In a preferred embodiment said nucleic acid comprises nucleic acid of a Semliki Forest Virus, a pox virus, a herpes virus and/or an adenovirus. In a preferred embodiment said nucleic acid delivery vehicle is a Semliki Forest Virus particle, a pox virus particle, a herpes virus particle or an adenovirus particle.

In another embodiment the invention provides a method for vaccinating an animal to obtain therein an immune response against at least one antigen, comprising administering sequentially to said animal, at least two different vaccine compositions, each containing at least said antigen or a precursor thereof and wherein at least two of said vaccine compositions differ from each other by the presence therein of a different vector. Preferably said animal is a human.

In yet another embodiment the invention provides a use of a vaccine composition in a method or a product of the invention.

In yet another embodiment the invention provides a use of an antigen, or a precursor thereof, for manufacturing a vaccine composition for vaccinating an animal or a human to obtain therein an immune response against said antigen, wherein said vaccine composition is administered sequentially with at least one other vaccine composition containing at least an immunogenic part, derivative and/or analogue of said antigen or antigen precursor, and a different vector.

As proof of principle we undertook a vaccine efficacy study comparing one vector system alone, two different combinations of two different vector systems, and the use of three different vectors administered sequentially. All